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(71) Applicant *(for all designated States except US)*: ALMQVIST & WALLENBECK AB [SE/SE]; Bruksgårdarna 48, S-743 50 Vattholma (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: WALLENBECK, Anders [SE/SE]; Bruksgårdarna 48, S-743 50 Vattholma (SE).

(74) Agent: ALDENBÄCK, Ulla; Dr. Ludwig Brann Patentbyrå AB, P.O. Box 1344, S-751 43 Uppsala (SE).

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(57) Abstract

The present invention relates to a probiotic composition comprising spores from one more strains of the yeast species *Endomyces fibuliger* (also named *Saccharomyces fibuliger* etc.). The composition can be used, on one hand, as feed additive to mammals (mainly monogastric ones), avian and fish for better growth and feed utilization and, on the other hand, as pharmaceutical/naturopathic preparation for human and animals, avian and fish, in particular against gastrointestinal disorders. Further, the composition can be used for controlling food intake. In addition, the composition can be used in order to inhibit putrefaction and mould growth, respectively.

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Title: Probiotic composition and the use thereof

Field of technology

The present invention relates to a probiotic composition comprising spores from one or more strains of the yeast species *Endomyces fibuliger* (also named *Saccharomyces fibuliger* etc.). The composition can be used, on one hand, as feed additive to mammals (principally monogastric), avian and fish for improved growth and feed utilisation and, on the other hand, as pharmaceutical/food/feed preparation for human and animals, avian and fish, especially against gastrointestinal disorders. Further, the composition can be used to control the intake of food. In addition, the composition can be used to inhibit putrefaction and mould growth, respectively.

Background of the invention

It is known that certain microorganisms, e.g. different lactic acid bacteria, have a favourable effect on the gastrointestinal flora of humans and animals. Microorganisms can be added either by fermented products, such as youghurt containing living cells (to human) and grass ensilage (to animals), or in a pure culture as probiotic preparation. The microorganisms of the fermented products die during the storage of the product and, therefore, the dose of living organisms becomes low and unreliable. The metabolites in the form of e.g. carboxylic acids and enzymes etc. in the fermented product may often be of greater importance for the aspects of food regarding health and assimilation of food than the occurrence of living cells.

When dosing microorganisms in pure culture per individual and day, the intention is to affect and make use of the fermentation process of the gastrointestinal tract in order to reduce disorders and to increase the utilisation of food. Organisms added shall i.a. suppress the activity of undesired organisms in the process. A probiotic product is intended to affect directly the fermentation process of the gastrointestinal tract. It is quite

another thing to process the food with the same organism to a certain quality before consumption, which is made e.g. when preserving and fermentering food and feed, compared to not allowing the organism to metabolize the food until in the gastrointestinal tract.

Appropriate probiotic microorganisms added with the food can i.a. influence the composition of bacteria and, owing to that, i.a. the enzyme activity and the fermentation in the gastrointestinal tract. The favourable microorganisms reduce disturbances from pathogenic microorganisms by suppressing the undesired organisms in different ways.

In order to reduce the occurrence of pathogenic organisms in animal production, antibiotics are often added in low doses. The problem of resistance development of pathogenic organisms and the transfer of the antibiotic substances into animal foodstuffs makes it very desirable to reduce the use of antibiotics to animals as well as humans. However, most of the known probiotic preparations have had difficulties in competing with antibiotic substances.

It is known that the animals' utilisation of food and their health condition are improved when adding suitable feed-decomposing enzymes. The choice of dose and type of enzyme is adjusted to the kind of animal and raw materials present in the feed. It is of great economical importance in animal production that the macro- nutrients for conversion of energy and building-up of protein are well utilised. The viscosity problem in the intestine often means that the species-specific enzymes do not manage to digest the food sufficiently. The problem also is that the individual does not himself produce the proper enzymes for effective digestion of the food. With the addition to the feed of specific enzymes and/or microorganisms supplementing the species-specific enzyme activity, a more effective utilisation of the energy and protein in the food can be achieved.

Many animal species have a high ability to consume feed. This often leads to overconsumption when the feed is supplied *ad libitum*. Then, the supply of feed has to be controlled mechanically to a restricted supply at several occasion per day. When there is a great consumption of feed at a few occations, the need of withdrawing heat arises. Heat development occurs when the food is decomposed. The quality of e.g. pork meat is dependent on i.a. the fat provision. This, in turn, depends on the amount of food consumed per day. At present, there are only mechanical methods at disposal in order to restrict the eating amount per day.

The use of the yeast *Endomyces fibuliger* in a highly vital spore culture as probiotic composition is not previously known. This yeast species is known for use in the fermentation of i.a. starch-rich foodstuff raw materials, mostly in certain Asian countries. The yeast is known, to a lower extent, for the fermentation of feed and for other specific purposes, e.g. for making use of starch-rich waste in the agricultural processing industry for the manufacture of feed protein in the form of killed yeast (e.g. the "symba" process where organisms of the species *Endomyces fibuliger* are used to digest the starch contents of the waste to sugar and *Saccharomyces cerevisiae* is used for the growth of the single cell protein).

In *Mikrobiologigiya i bioteknologiya proizvodstva kormov*, pages 93-106, 1990 there is disclosed an experiment where swine are fed with cereal (barley) and straw fermented with the yeast species *Endomyces fibuliger*, increasing the daily body weight and the feed utilisation of pigs, compared to feed that had not been fermented. These effects are achieved with a high proportion of fermented feed in the feed ration >50%. There is no mention of any specific coupling to the activity of the yeast cell in the gastrointestinal tract.

In *Applied Microbiology*, October 1973, pages 650-652, Kurtzman et al. disclose that *Saccoromycopsis (Endomycopsis) fibuligera* produces a mixture

of C_{14}-C_{18}-hydroxy fatty acids, which mixture has antimicrobial activity against *Vibro trognes* but not against any other microorganisms tested. There is no mention of the use of the yeast for better feed utilisation and as a probiotic.

In Japan there is sold a natural product, BX-1, since mid 1980th. It is produced by fermenting rice bran with a mixed culture of an originally natural contamination of a number of different organisms, of which *Bacillus brevis* and a number of different strains of *Endomyces fibuliger* are predominant. The interesting substances of the natural product are chiefly supposed to be residual enzymes of the fermented and dried bran. The natural product is used i.a. as feed additive, with good results on meat quality, animal health and feed utilisation. The natural product contains a very low number of living microorganisms compared to a highly active culture. The product is added to the animal feed in an admixture of 0.5-1.0%. The product is manufactured by composting rice bran where one intentionally, by using a low exposure to oxygen (meter-high composts) and a long time, seeks to reduce the number of living yeast cells in the fermented product. High occurrence of living yeast cells has been considered to be a problem as feed additive and, therefore, the composting process for the manufacture of the natural product has been adjusted so that the yeast is "unfairly treated" during the process.

A highly vital probiotic microorganism surviving the passage to the gastrointestinal tract and which is only needed in a low dose would be desirable. Apart from the fact that suitable probiotic candidate organism shall have a desired effect e.g. on health condition and the food utilisation, there must be a rational way of distributing the probiotic culture to the gastrointestinal tract. When distributing via dry feed, it is required that the culture manages to survive both the heating when pelleting the feed and the air oxygen during the storage up to consumption.

For the distribution via dry feed, cultures would be desirable which are thermostable (withstand high temperatures), oxygen-stable (withstand O_2) and acid-stable (withstand high concentrations of hydrogen ions H^+) which would solve two important problems. One problem is the difficulty for the animal producer himself to distribute 1000th part amounts via daily feed processing. Industrial premixture in premixes of probiotic preparations in ppm amounts solves the problem of achieving a continuous dosing. The other important problem is that many microorganisms do not survive the stomach and therefore do not reach the intestines in a vital condition.

Summary of the invention

The object of the present invention is, therefore, to provide a probiotic composition providing reduced incidence of gastrointestinal disorders in humans and animals as well as increased feed conversion and possibility of feed control in animals.

According to a first aspect, the invention relates to a probiotic composition comprising spores from a highly vital culture selected from suitable strains of the yeast species *Endomyces fibuliger* (also named *Saccharomyces fibuliger* etc.).

The yeast species used according to the invention is preferably prepared by the solid fermentation method disclosed in Applicant's SE application No. 9800506-9 filed simultaneously and which hereby is included by reference. This method provides ae highly vital spore culture (ascospores) which is thermostable, oxygen-stable and acid-stable.

Endomyces fibuliger is a harmless yeast species which is commercially available in different oriental starter cultures for the fermentation of starch-rich foodstuffs. E.g. strain ATCC 2080; Chinese yeast for the preparation of "hoang-chiu", strain ATCC 24945; "Ragi & Tape" fermentation, strain ATCC

32693; Thailand, for the production of "lookpang" (similar to Ragi), strain ATCC 46252; Taiwan, for the preparation of "lao chao", strain ATCC 46253; Taiwan, production of "tape ketan", strain ATCC 46949; commercial "ragi-starter", strain ATCC 52921; the Philippines, production of "tapuy", strain ATCC 60235 Taiwan, food fermentation. The yeast species is also contained i.a. as one of two yeast species in the manufacture of the Japanese national drink Zake (*Amazake).*

The normal dose of known probiotic cultures is about 500 000-1 000 000 cfu/g (colony-forming cells per gram) ds (dry substance) feed as prophylaxis. According to the invention, a suitable dose of *Endomyces fibuliger* as prophylaxis is about 5000-100 000 cfu/g ds feed, e.g. 5 000-10 000 cfu/g ds feed.

According to a second aspect, the invention relates to a composition comprising *Endomyces fibuliger* as a pharmaceutical preparation. The pharmaceutical preparation is mainly intended for treatment of gastrointestinal disorders in humans and animals. In prophylactic treatment of animals, the composition is contained in feed and has an activity of *Endomyces fibuliger* of 5000-100 000 CFU/g ds feed. In therapeutical treatment of gastrointestinal disorders in humans and animals, the composition is administered in a daily dose of 10^7-10^{13} CFU/individual.

Further aspects of the invention are i.a. the use of the *Endomyces fibuliger* composition according to the above for promoting feed conversion and daily growth in animals, control of food intake, inhibition of putrefaction and inhibition of mould growth, respectively.

A surprising discovery in tests carried out for feeding swine by addition to the feed of the yeast *Endomyces fibuliger* is that the daily consumption of feed decreases with increased dose. An important part of the increased feed

conversion is explained by the fact that the animals change their eating behaviour to lower amounts per eating occasion and that they digest the food during a major part of the day. The energy losses due to heat diversion decrease and the animals eat less and grow faster at a balanced low-dose level. Thus, by increasing or reducing the dose of *Endomyces fibuliger* one can increase or decrease the daily consumption.

At yeast doses of 50 000 cfu/g ds feed to swine, tests carried out show 12% lower daily consumption compared to 5 000 cfu/g ds feed. With regard to swine breeding one can thus optimize the feed consumption with regard to the quality desired (e.g. fat provision & meat percentage in the slaughtering body) and adjust the yeast dose to divergences of female and male pigs and castrated pigs.

Endomyces fibuliger does not colonize in the gastrointestinal tract but probably it manages to germinate from the spore and propagate a few times at the passage. It can grow in the intestine but probably slowly. However, for an optimal growth environment much more and easily available oxygen is required. *Endomyces fibuliger* is, in living form when growing, a very large organism. The typical thing is that it grows to a bush-like collection of yeast cells where the cells, to begin with, are more similar to fungus hyphae than to the form and size of the typical yeast cell. It takes the typical shape of the yeast cell, monocellular and large with an oval-shaped form when the colony grows older at decreasing growth. The size and the form may partly explain the low dose requirement owing to the fact that the production of metabolites takes place in a large biomass relative to the number of organisms. The yeast spores added to the feed are, however, very small and can be compared to the size order of the bacterium.

The living yeast *Endomyces fibuliger* withstands very low pH values for a long time. The yeast effectively produces (aerobically as well as unaerobically)

acetic acid and lactic acid. Due to the fact that it is a hyper-producer of amylases, these bacteriostatic carboxylic acids are produced directly from a starch substrate. The distribution between the production of acetic acid and lactic acid varies from strain to strain but the amount is often comparable. It is known that a mixture of these acids has a very good bacteriostatic effect. The yeast also produces other bacteriostatic carboxylic acids in small amounts as well as smaller amounts of alcohol. The yeast inactivates bacteria but also fungi, in addition to the bacteria which do well in the acidic environment which the yeast establishes quickly. The dosage and nutrient access of the yeast are decisive for the bacteriostatic effect in the intestine during the passage of food in the gastrointestinal tract. *Endomyces fibuliger* is an extreme O_2-consumer. This, to a certain extent, can contribute to creating favourable conditions for growth of anaerobic lactic acid bacteria. A good establishment of lactic acid bacteria is important for the outer protective function barrier of the mucose.

According to the invention, *Endomyces fibuliger* can be used *per se* or in combination with other organisms known to have probiotic effect in mixed cultures. One requirement is that they "do well" together with the yeast. Spore cultures of different *Bacillus* species, such as *B. cereus, B. subtilis, B. brevis* etc., are of particular interest in distribution via dry feed, which cultures withstand heating in feed processing and oxygen exposure during the storage of the feed up to consumption. Coated or encapsulated cultures of other medically or probiotically beneficial organisms are also of interest. The production of spore cultures of *Bacillus* species does not require the use of any expensive processing method for coating of the organism in order to withstand heat and the air oxygen. Several different strains of *Bacillus* species are today approved as probiotic feed additives. Together with *Endomyces fibuliger* there is obtained, with *Bacillus* strains, a broader spectrum of the enzyme production in the fermentation of the intestine. Observations indicate that a full synergistic effect probably is achieved with

extremely low maintenance doses of *Bacillus* spores; down to one hundredth of the normal probiotic dose for prophylaxis. *Endomyces fibuliger* seems to create particularly favourable conditions in order for these *Bacillus* strains to do well in the intestinal tract.

The addition of *Endomyces fibuliger* in monoculture and in co-culture with *Bacillus brevis* has, in experiments, resulted in a reduced incidence of diarrhoea in swine and an increased feed utilisation. The swines' hunger becomes more satisfied and they stop being compulsive eaters with the addition of *Endomyces fibuliger* in the feed of the invention. The animals digest the food for a longer time of the hours of the day. A more distributed eating and a longer time for digestion result in a more effective economizing of the food energy. A concentrated eating at a few occasions results in that the animals have to cool-off the heat excess when the larger amount of food per eating occasion is digested. Then, the animal body is forced to consume energy from the body reserve (resulting in further losses) when the digestion of food moves "into a low gear" before the next eating occasion.

It was striking that the lower cooling need of the treatment group resulted in that they consistently kept drier and, owing to that, cleaner on the body. This is explained by the fact that they did not find their way to the manure part in order to roll themselves, as much and as often, to cool down themselves after feeding.

The eating behaviour seems to change so that the pigs eat less on each occasion and eat on several occasions. Usually, the feeding of swines takes place on three occasions per day. The eating takes place under great competition within the litter, where the strongest ones eat more at the expense of the weaker individuals. The changed eating behaviour gives rise to two effects. The one effect is that the daily consumption per pig decreases at the same time as growth increases. The other effect is that the competition

within the litter of the food is reduced by extended eating times which results in a more even intake of feed and growth in litters. The daily consumption decreases with increased dose of the yeast *Endomyces fibuliger* and, therefore, overdosing of the yeast leads to an economically unoptimal daily growth of the swines.

Consequently, the effect of *Endomyces fibuliger* as feed additive is, on one hand, to be probiotic with a reduced incidence of diarrhoea and, on the other hand, to influence the decomposition process of the feed favourably with regard to the utilisation of the nutrient of the food with regard to energy as well as protein.

Detailed description of the invention

In animal experiments, very satisfactory probiotic effects have been achieved with a dose as low as about 5 ppm dried spore culture with 10^9 cfu/g corresponding to a dos of 5000 cfu/g of *Endomyces fibuliger* per gram feed dry weight. When treating certain diarrhoea at indication, a quicker and better result is obtained at an increased dose, 10-100 times or more.

The results of feeding experiments confirm that the feed utilisation increases essentially. This can not be explained only by differences in the probiotic effect by reduced incidence of gastrointestinal disturbances. The moderating effect of the yeast, with increased dose, on the rate of the fermentation in the intestine as well as the faster digestion of the starch to simple sugars by the hyperproduction of amylases of the yeast is probably the explanation of changed eating behaviour and a more effective balance during the day in order to utilize the energy of the food. The utilization of the food's contents of amino acids increases at the level of increased energy utilization or more. This can be due to the fact that the process of putrefaction in the intestine seems to decrease. The probable explanation is that a suppressed fermentation rate in the intestine results in that the proportion of free amino

acids managing to be broken down into simpler constituents decreases and the proportion taken up by the intestine increases.

Experiments with *Bacillus brevis* in co-culture with *Endomyces fibuliger* show that, during the growth phase in starch, the population of *B. brevis* growths very quickly. This is probably due to the fact that *E. fibuliger*, on one hand, quickly releases i.a. simple sugars from the starch and, on the other hand, creates exceptionally favourable life conditions for lactic acid-producing organisms in that *E. fibuliger* rapidly reduces the pH and effectively consumes free O_2. When the digested food reaches the intestine, this means that naturally occuring lacto- bacteria in the intestine and other bacteria added as probiotics probably are provided with very good conditions for growth and establishment when present in the environment created by *E. fibuliger*.

This may explain why the dose level, in prophylaxis with probiotic bacteria establishing in the gastrointestinal tract, probably can be reduced in mixed culture with *E. fibuliger*, without loss of effect.

Example 1: Dysentery in pigs treated with high doses of *Endomyces fibuliger*

20 weaned piglets fallen seriously ill in dystentery (live weight about 15 kg) were collected in two boxes among about 400 piglets without symptoms, in a major stock. They were given a daily high-dose of 10^9 cfu *E. fibuliger*. 19 out of the 20 piglets became asymptomatic after 2-3 days. The treatment was stopped after 7 days, the symptoms then recurred. On day 15 the treatment was reintroduced and, once again, the pigs became asymptomatic within 2-3 days. The high-dose treatment continued up to day 28, whereupon a low-dose 10^4 cfu/g was mixed into the feed to 25 kg live weight. The symptoms did not recur.

Example 2: Diarrhoea in pigs treated with high doses of *Endomyces fibuliger*

In this experiment, the normal treatment with "Tylan" at the diarrhoea indication was replaced, during the period from weaning up to delivery at 25 kg live weight, with a high-dose 10^9 cfu/day of *Endomyces fibuliger*, given orally, with a result comparable to the "Tylan" therapy. In additional experiments it could be noted that, in some cases where the "Tylan" treatment did not provide any effect, *Endomyces fibuliger* was efficient and *vice versa*.

Example 3: Prophylactic treatment against diarrhoea in pigs with a low dose of *Endomyces fibuliger*

In this experiment, low-dose ration was set in with 10^4 cfu/g of *E. fibuliger* in the feed, to a group of 200 piglets, and was compared to a control group of the same size. The diarrhoea incidence in the control was kept normal for the stock about 20% from the weaning up to 25 kg live weight but was reduced drasticly in the test group to a few single cases.

Example 4: The growth of pigs is influenced by a high dose of *Endomyces fibuliger*

In a fourth experiment, the feed dose was increased 10 times to a group of 400 piglets. The high-dose experiment was interrupted after four weeks since it was observed that the growth became low. The health study continued with the low-dose ration in the feed. The growth returned to normal growth immediately upon the decrease of the dose and the health status was excellent at the introductory high-dose as well as at the subsequent low-dose prophylaxis.

Example 5: Feed control with *Endomyces fibuliger*; specific dose (kind of animal, age, sex)

In another stock, 220 slaughter swines were given, to begin with, an additive of 10^4 cfu/g of *E. fibuliger* in the feed from 25 kg live weight. No health disturbances occured up to slaughter except from one pig showing an indication of diarrhoea on one occasion. In the experiment, the effect on growth and consumption was studied by increasing the dose 10 times to 10^5 cfu/g feed. At the increase, which was made at about 65 kg live weight, the daily consumption immediately decreased with 0.3 kg (about 12%) to 2.5 kg feed/day. The feed intake returned to the normal one directly when the dose, after some weeks, was reduced to 10^4 cfu/g feed. The lower growth at the high-dosing of *E. fibuliger* was directly proportional to the lower feed intake, compared to the low-dose 10^4 cfu/g feed.

The results of this experiment indicate that the *E. fibuliger* composition according to the invention might have a potential effect as a weight reducing preparation also for humans.

Example 6: Indigestions in humans treated with *Endomyces fibuliger* in high dose

A person with lingering stomach problems having been treated in 1994 for a very long time with different antibiotics, became quite asymptomatic within 2 days when setting in treatment with *E. fibuliger* in a dose of 1 g or 10^9 cfu/day. The treatment went on for two weeks and the symptoms did not recur after completed treatment.

Several other volunteeres have tested the yeast for ordinary stomach problems (ordinary diarrhoea) and all of them have been reported to recover rapidly. One person being treated with (omeprazol) (Losec®) with no effect,

became asymptomatic within 2 days. Diarrhoea and stomach pain ceased within 1-2 days. It has also been reported that they got rid of previously recurring problems in spite of the fact that they did not continue to "eat the yeast" which indicates that the normal function of the intestinal flora probably may have been restored by the yeast.

These observations on human have the same lapse of time as observed for the treatment of diarrhoea in swine.

Example 7: Diarrhoea in sucking-calves treated with *Endomyces fibuliger*

In this experiment, the effect on sucking-calves was studied. Positive effects on the incidence of calf diarrhoea was not expected and they also were absent. Lactic sugar inactivates the yeast, in addition the yeast probably requires starch-rich nutrient solution with high contents of fiber in order that the metabolism shall "balance-in" at high production of lactic acid and acetic acid.

Example 8: Diarrhoea in milk cows treated with *Endomyces fibuliger*

A whole stock of about 280 milk cows got diarrhoea with symptoms similar to viral diarrhoea. There was tested whether the yeast *Endomyces fibuliger* could help, however there was no expectation of any effect. 5000 cfu/g dry feed substance were distributed to the whole stock. The diarrhoea disappeared at the same time in all cows within 3 days. The excrement became more solid than before the cows fell ill. The milk production which decreased with more than 30% when the cows fell ill returned to normal level within a week and continued to increase thereafter. After 5 weeks' low-dose treatment the experiment was interrupted. The reason for the interruption was that whole kernels of lye-treated wheat passed the

gastrointestinal tract without being digested. The yeast inhibited the cellulase-active organisms of the rumen. Immediately upon removal of the yeast, the phenomenom of whole wheat kernels in the faeces disappeared. The balance was restored in the rumen to high cellulase production and the milk production continued to increase over levels achieved before the diarrhoea outbreak. These observations confirm hyper-production of amylase of *E. fibuliger* in the anaerobic environment of the rumen.

Example 9: *Endomyces fibuliger* inactivates putrefactive bacteria

Nutritive substrate in the form of starch-rich foodstuff products were infected with microorganisms from excrements, rumen liquid etc., natural flora in stored cereals etc., with and without the presence of *Endomyces fibuliger.*

With the addition of *Endomyces fibuliger*, similar results were obtained in all cases, that is, the process of putrefaction ceases or is suppressed very rapidly and effectively, even at good oxygen supply to the substrate. The yeast species *Endomyces fibuliger* immediately takes control over the fermentation and the typical odour of putrefaction disappeared or was reduced drasticly in aerobic environment as well as in anaerobic environment. This aspect of the invention is useful for e.g. reducing putrefaction of manure in basins and compost.

Example 10: *Endomyces fibuliger* inhibits mould growth

In tests it has been observed that mould fungus can not grow in a specimen with metabolizing *Endomyces fibuliger.* However, when the nourishment runs out so that *E. fibuliger* no longer can metabolize and the nutritive substrate has been allowed to stand and evaporate for a long time, mould spores will be able to grow. Consequently, there are volatile substances

which provide 100% inhibition of mould growth in nutritive substrates where *E. fibuliger* metabolizes. This aspect of the invention is useful for e.g. preserving foodstuffs and cereals.

FEEDING EXPERIMENTS WITH CONTROL

These experiments will be described with reference to the enclosed drawings wherein

Fig. 1 shows the consumption in kg feed per kg weight gain live weight (FCR = feed conversion rate) and percentage improvement in test group compared to control group;

Fig. 2 shows the average daily weight gain (DWG = daily weight gain) and percentage increase in test group compared to control group;

Fig. 3 shows the average daily feed intake (DFI = daily feed intake);

Fig. 4 shows the number of test days in respective experiments;

Fig. 5 shows a summary of Fig. 1-4 i tabular form; and

Fig. 6 shows live weight at onset and termination, respectively, for each experiment.

Example 11a: Weight gain per kg feed (FCR) in slaughter swines treated with *Endomyces fibuliger* and *Bacillus brevis* (Fig. 1-6, trial No. 2)

A group consisting of 82 slaughter swines was treated by addition to the feed of a culture comprising spores, on one hand, from the species *Endomyces fibuliger*, dose 5000 cfu/g feed (dw), and, on the other hand, from the species *Bacillus brevis*, dose 2000 cfu/g feed (dw). The control consisted of 83 pigs.

The feed consumption decreased from 3.35 kg feed/kg growth to just below 2.98 kg feed/kg growth (live weight, lw) for a 20 days test period (Fig. 4). The slaugther swines in the treated group ate 4.9% less feed per day (Fig. 3) and

grew 7.1% more rapidly (Fig. 2). Therefore, the feed conversion was improved with 11% (Fig. 1) in the treated group compared to the control.

The difference in the result of the test group compared to the result of the control group was very distinct.

Example 11b and c: Weight gain per kg feed (FCR) in slaughter swines treated with *Endomyces fibuliger* (Fig. 1-6, trials Nos. 3 and 4)

In these two experiments, the feed was provided with only yeast spores of the species *Endomyces fibuliger*, dose 5000 cfu/g feed (dw). In trial No. 3, a restrictive ration was given with the same amount of feed per day to both the control and the treated group; in trial 4 feed was provided *ad libitum* up to about 65 kg live weight.

The improvement of the feed conversion (FCR Fig. 1) became 6.5% in trial 3 and 5.6% in trial 4. In trial 3, the animals grew 6.9% more rapidly and in trial 4 they grew 1.9%. The feed consumption was the same in the two groups in trial 3 and 3.8% lower per day for the treated group in trial 4, compared to the control. Thus, the feed conversion rate (FCR) was improved almost just as much with the addition of the composition in trials 3 and 4 with very different daily growth and feed consumption. This is in correspondence with the explanation given and therefore the experiments do not contradict the fact that the consumption, in proper dose, may lead to less heat losses and better economization of the nutritive contents of the food.

Example 11d: Weight gain per kg feed (FCR) in piglets treated with *Endomyces fibuliger* (Fig. 1-6, trial 5)

In this controlled experiment with piglets (127+126 individuals), the feed was supplied only with yeast spores of the species *Endomyces fibuliger*, dose 5000 cfu/g feed (dw). The improvement of the feed conversion (FCR Fig. 1) became 10.6% and the daily growth (DWG Fig. 2) was improved with 7.7% whereas the feed consumption (DFI Fig. 3) decreased with 3.3% in the treated group in trial 4, compared to the control.

FEEDING EXPERIMENTS WITHOUT CONTROLS

Example 12: Improvement in FCR and reduction of the number of feeding days in slaughter swines treated with the addition of a low dose of *Endomyces fibuliger* in feed

In trial 1, 5000 CFU/g of *Endomyces fibuliger* were given in feed to 10 000 slaughter swines from onset weight to slaughter. The trial was conducted for a two years period at Trial Farm 2 (the controlled experiments are accounted for in Figures 1-6). The production efficacy of the slaughter swine breeding for the two years (about 10 000 slaughted pigs) was compared to the two previous years. The improvement of feed yield (FCR) was about +13% which resulted in a reduction of the number of feeding days with -8%. The difference in productivity in breeding before and after the time for addition of yeast spores to the feed is indicative. However, they are consistent with the results from the controlled experiments. The yeast strain as additive to the feed seems to play an important role as a probiotic and growth-improving microorganism.

Summary

To summarize, the feeding experiments show an essential improvement of feed conversion and daily growth and a small but important reduction of the daily feed intake when *Endomyces fibuliger*, with or without *Bacillus brevis*, is added to the feed.

These studies show that an extremely low dose in cfu/g feed of *Endomyces fibuliger* has an essential probiotic effect on the animal health and the efficacy of the feed conversion. In addition, it is clearly shown that the feed additive according to the invention reduces the daily feed intake.

CLAIMS

1. Probiotic composition, characterized by comprising spores from one or more strains of the yeast species *Endomyces fibuliger* (*Saccharomyces fibuliger*) producing high contents of lactic and/or acetic acid as well as high contents of amylase.

2. Probiotic composition according to claim 1, characterized by comprising feed additives.

3. Probiotic composition according to claim 1 or 2, characterized by comprising, in addition, other spore-forming or coated organisms having probiotic activity.

4. Probiotic composition according to claim 3, characterized in that the spore-forming organisms are *Bacillus* species.

5. Composition comprising spores from one or more strains of *Endomyces fibuliger* (*Saccharomyces fibuliger*) for use as a pharmaceutical preparation for humans and animals.

6. Composition according to claim 5, characterized by comprising, in addition, other spore-forming or coated organisms having medical activity.

7. Composition according to claim 6, characterized in that the spore-forming organism is a *Bacillus* species.

8. Use of the composition according to claims 5-7 for the preparation of a pharmaceutical preparation for prophylaxis and therapy against gastrointestinal disorders in humans and animals.

9. The use of a composition according to any of claims 1-4 for promoting feed conversion and daily growth of animals.

10. Use of a composition according to any of claims 1-4 for controlling feed intake, the feed intake being reduced when the dose of the composition increases and *vice versa*.

11. Use according to claim 9 or 10, characterized in that the composition is contained in feed and has an *Endomyces fibuliger* activity of 5000-100 000 CFU/g dry feed substance.

12. Use of a composition according to any of claims 1-4 for inhibiting putrefactive organisms.

13. Use of a composition according to any of claims 1-4 for inhibiting organisms causing mould growth.

Figure 1

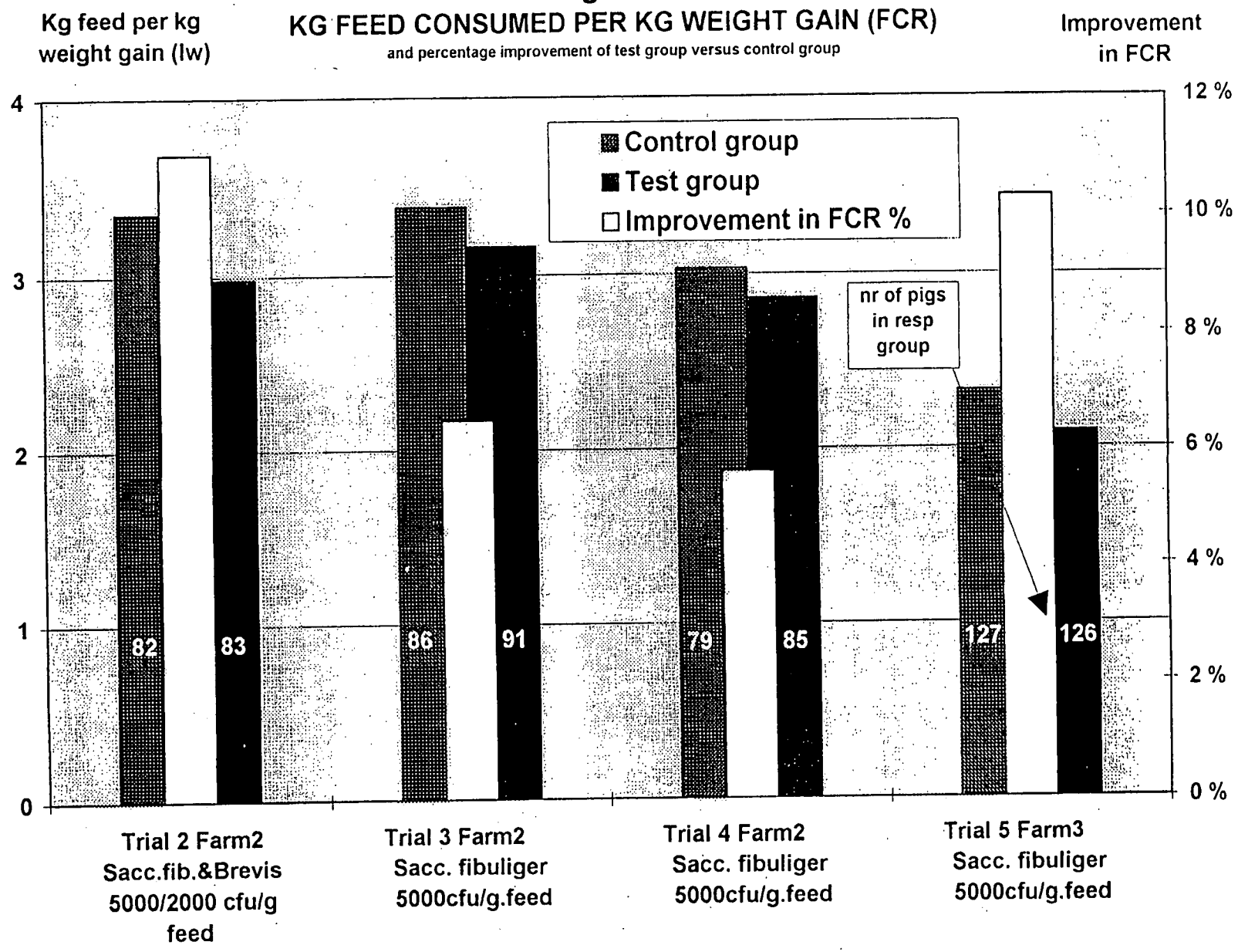

KG FEED CONSUMED PER KG WEIGHT GAIN (FCR)
and percentage improvement of test group versus control group
Kg feed per kg weight gain (lw)
Improvement in FCR
Control group
Test group
Improvement in FCR %
nr of pigs in resp group
4
3
2
1
0
12 %
10 %
8 %
6 %
4 %
2 %
0 %
82
83
86
91
79
85
127
126
Trial 2 Farm2 Sacc.fib.&Brevis 5000/2000 cfu/g feed
Trial 3 Farm2 Sacc. fibuliger 5000cfu/g.feed
Trial 4 Farm2 Sacc. fibuliger 5000cfu/g.feed
Trial 5 Farm3 Sacc. fibuliger 5000cfu/g.feed

Figure 2

DAILY WEIGHT GAIN (DWG)

and percentage improvement of test group versus control group

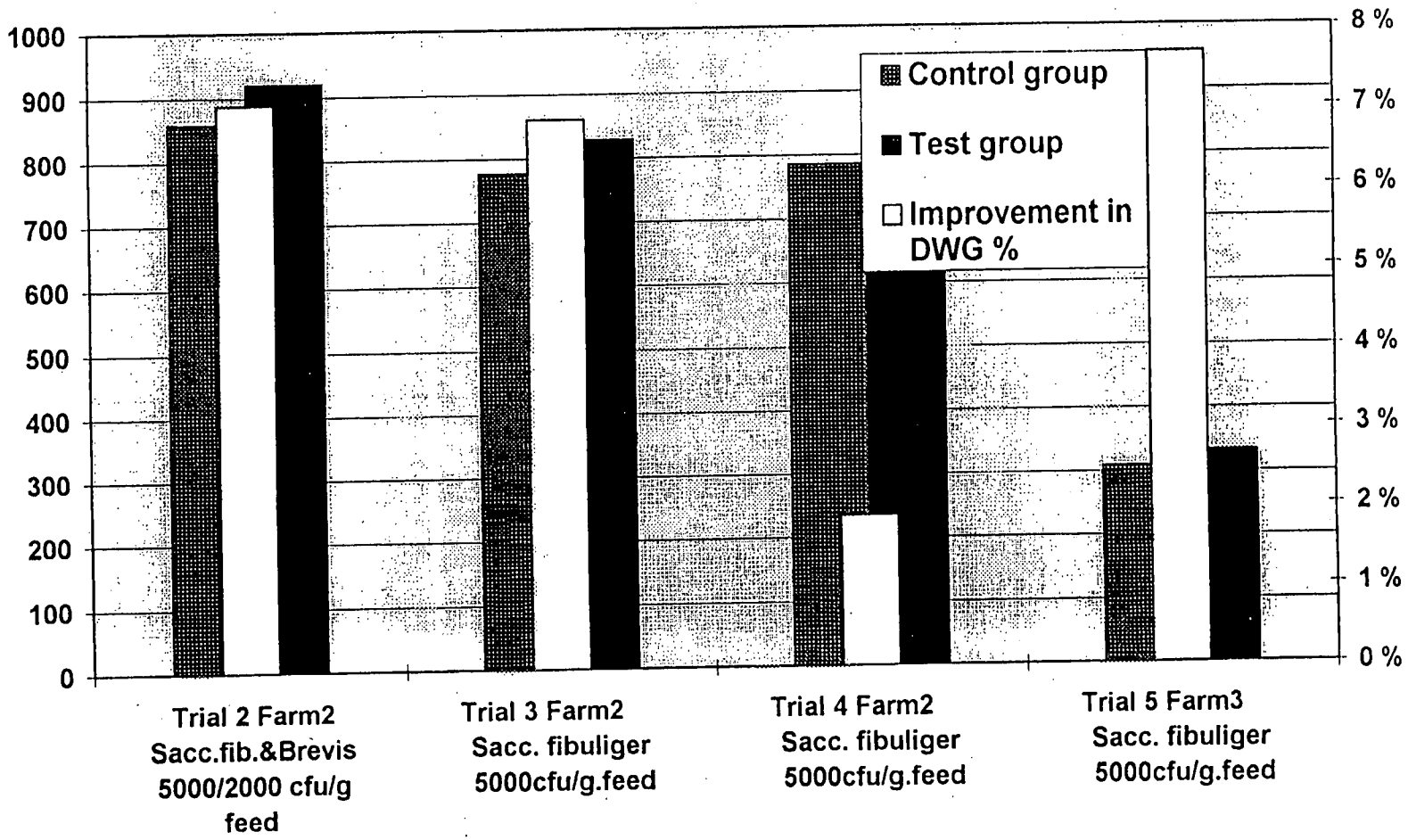


Figure 3

MEAN DAILY FEED INTAKE (DFI)

and percentage decrease of test group versus control group

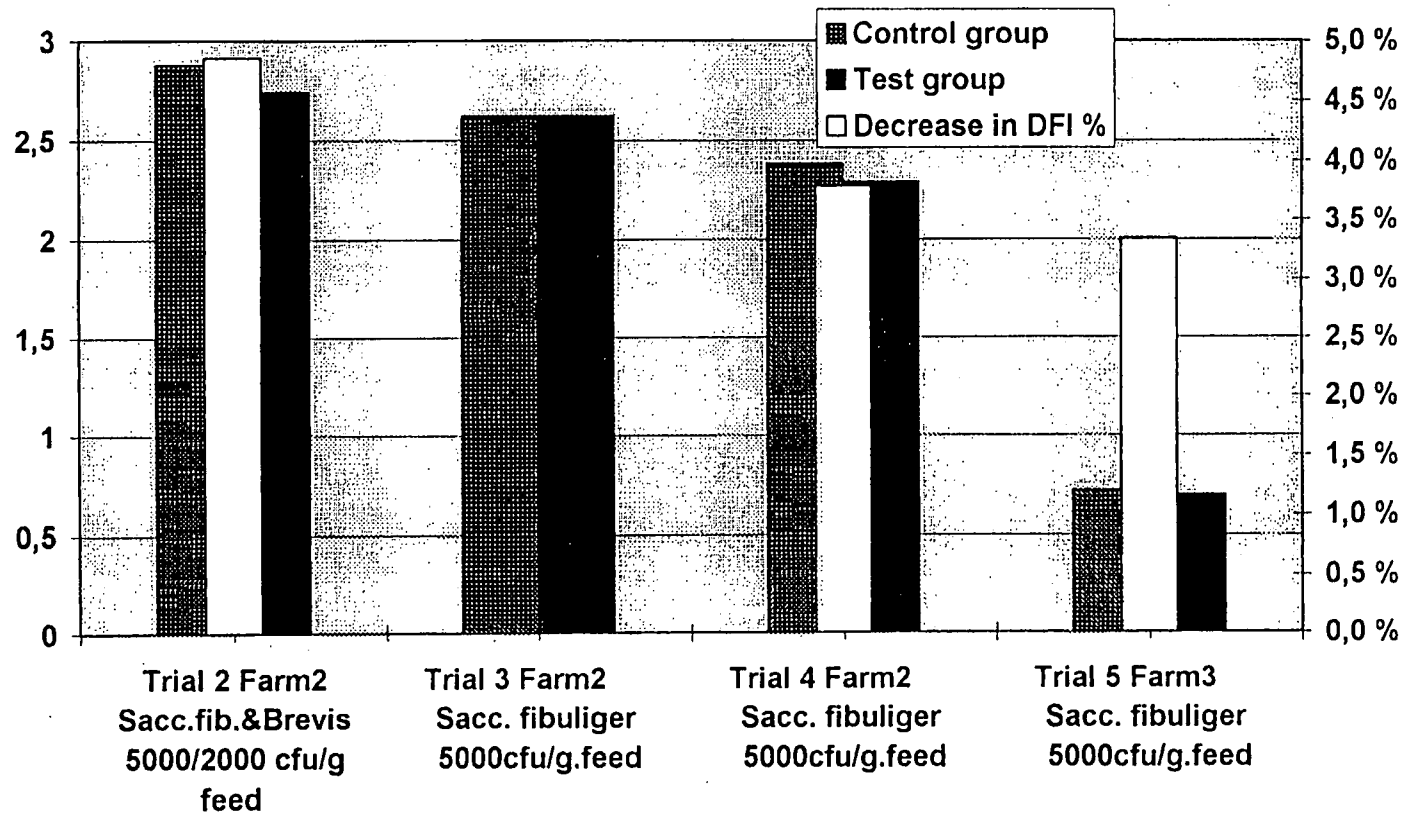


Figure 4
MEAN DAYS ON TEST

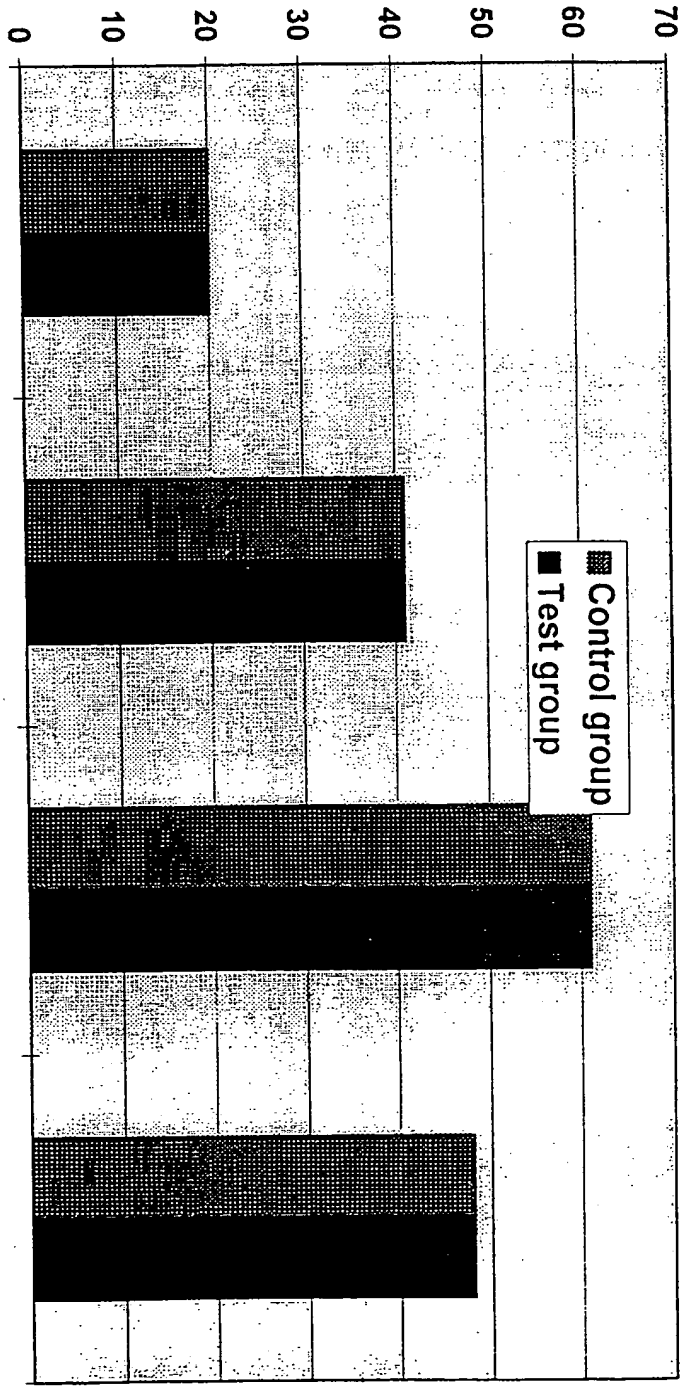


Figure 5

FIGURE 1

TRIAL	Control group	Test group	Improvement in FCR %
Trial 2 Farm2 Sacc.fib.&Brevis 5000/2000 cfu/g feed	3,35	2,98	11,04
Trial 3 Farm2 Sacc. fibuliger 5000cfu/g.feed	3,38	3,16	6,51
Trial 4 Farm2 Sacc. fibuliger 5000cfu/g.feed	3,03	2,86	5,61
Trial 5 Farm3 Sacc. fibuliger 5000cfu/g.feed	2,33	2,09	10,30

FIGURE 2

TRIAL	Control group	Test group	Improvement in DWG %
Trial 2 Farm2 Sacc.fib.&Brevis 5000/2000 cfu/g feed	858,5	919,5	7,11
Trial 3 Farm2 Sacc. fibuliger 5000cfu/g.feed	775,6	829	6,88
Trial 4 Farm2 Sacc. fibuliger 5000cfu/g.feed	785,2	800	1,88
Trial 5 Farm3 Sacc. fibuliger 5000cfu/g.feed	308,3	331,9	7,65

FIGURE 3

TRIAL	Control group	Test group	Decrease in DFI %
Trial 2 Farm2 Sacc.fib.&Brevis 5000/2000 cfu/g feed	2,88	2,74	4,86
Trial 3 Farm2 Sacc. fibuliger 5000cfu/g.feed	2,62	2,62	0,00
Trial 4 Farm2 Sacc. fibuliger 5000cfu/g.feed	2,38	2,29	3,78
Trial 5 Farm3 Sacc. fibuliger 5000cfu/g.feed	0,719	0,695	3,34

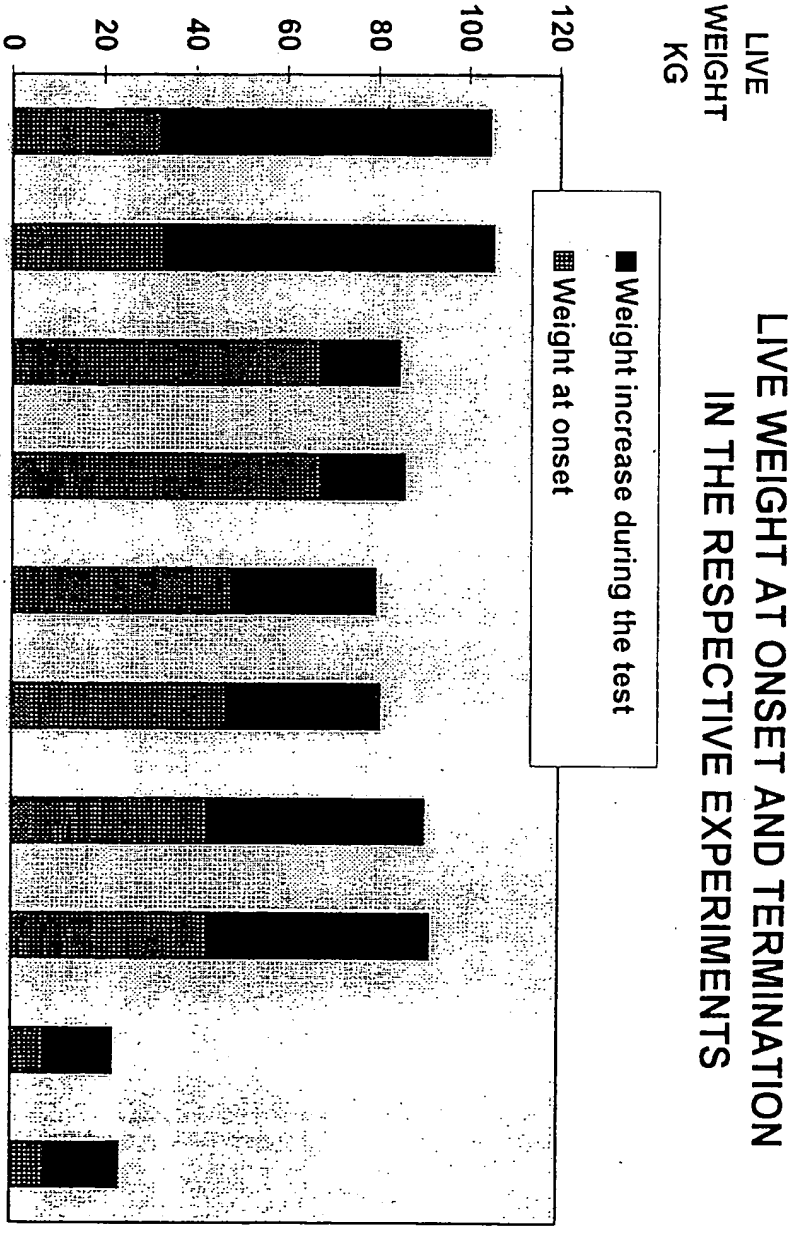
FIGURE 4

TRIAL	Control group	Test group
Trial 2 Farm2 Sacc.fib.&Brevis 5000/2000 cfu/g feed	20	20
Trial 3 Farm2 Sacc. fibuliger 5000cfu/g.feed	41	41
Trial 4 Farm2 Sacc. fibuliger 5000cfu/g.feed	61	61
Trial 5 Farm3 Sacc. fibuliger 5000cfu/g.feed	48	48

FIGURE 5

TRIAL	Weight at onset	Weight increase during the test
Trial 2 Farm 2 CONTROL GROUP	67,44	17,17
Trial 2 Farm 2 TEST GROUP	67,39	18,38
Trial 3 Farm 2 CONTROL GROUP	47,65	31,80
Trial 3 Farm 2 TEST GROUP	46,49	33,99
Trial 4 Farm 2 CONTROL GROUP	42,50	47,90
Trial 4 Farm 2 TEST GROUP	42,80	48,80
Trial 5 Farm 3 CONTROL GROUP	7,32	14,80
Trial 5 Farm 3 TEST GROUP	7,67	15,93

Figure 6
LIVE WEIGHT AT ONSET AND TERMINATION
IN THE RESPECTIVE EXPERIMENTS



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00231

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23K 1/16, A61K 35/72, C12N 1/16
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, A23K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, BIOSIS, MEDLINE, FOODSCI, BIOTECHNOLOGY ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	File WPI, Derwent accession no. 82-69779E, AS LATV MICROBIOL, "Animal feed microbial additive - contg. Endomicopsis fibuliger R-574 yeast strain biomass for glucoamylase and cellulase enzymic activity", SU,B,874022, 811025 --	1-13
A	File WPI, Derwent accession no. 85-268607, Biotech Res Inst: "Yeast strain Endomycopsis fibuliger C-4 - is used as producer of feedstuff protein"; & SU,A,1150264, 850415 --	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 April 1999	15 -06- 1999
Name and mailing address of the ISA Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Yvonne Siösteen Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00231

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Informatin Services, file 351, DERWENT WPI, Dialog accession no. 003233765, WPI accession no. 81-94323D/198151, AS LATV MICROBIOL: "Fodder additive contg. cellulase and glucoamylase enzymes - prepd. by jointly cultivating specified strains of fungus Trichoderma lignorum and yeast Endomycopsis fibuliger"; & SU,B,812258, 19810315 --	1-13
A	Dialog Information Services, file 155, MEDLINE, Dialog accession no. 07658723, Medline accession no. 94029535, Kmet V et al: "Probiotics and manipulation of rumen development and function"; & Arch Tierernahr, 1993, 44 (1) p1-10 -- --------	1-13